

Morphological and histological study of the scales of the grey notothen, *Lepidonotothen squamifrons* (Günther 1880) (Nototheniidae; Perciformes; Teleostei)

by

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Received: 31 Mar. 2018
Accepted: 18 Feb. 2018
Editor: O. Otero

Key words

Lepidonotothen
Scales
Histology
Mineralization
Cteni
Mandl's corpuscles

Abstract. – The scales of the grey notothen, *Lepidonotothen squamifrons* (Günther, 1880), show the classical organization of ctenoid scales: an external acellular ornamented bony layer bordered by posterior spines, the ctenii, and overlaying an incompletely mineralized basal plate. The basal plate is constituted by a number of collagenous layers that form an orthogonal plywood-like organization. The mineralization of the basal plate progresses due to the formation of ovoid Mandl's corpuscles that progressively fused to each other. The morphology and the histology of scales of the grey notothen are similar to the scales of numerous Perciformes, such as the Percidae and the Cichlidae for example.

Résumé. – Étude morphologique et histologique des écailles du colin austral, *Lepidonotothen squamifrons* (Günther, 1880) (Nototheniidae; Perciformes ; Teleostei).

Les écailles du colin austral, *Lepidonotothen squamifrons* (Günther, 1880), montrent l'organisation typique des écailles cténoïdes : une couche externe osseuse acellulaire ornementée, bordée postérieurement par une rangée d'épines, les ctenii. Cette couche externe recouvre une plaque basale lamellaire incomplètement minéralisée. La plaque basale de l'écaille est constituée de couches successives de fibres de collagène formant un contre-plaqué orthogonal. La minéralisation de la plaque basale progresse par formation et fusion de corpuscules de Mandl ovoïdes. La morphologie et l'histologie des écailles du colin austral sont donc très proches de celles des écailles d'autres Perciformes, comme les Percidae et les Cichlidae par exemple.

The grey notothen, *Lepidonotothen squamifrons* (Günther, 1880) (Nototheniidae), is a common fish on the shelf of Kerguelen and Crozet Islands (Austral Ocean). The family Nototheniidae belongs to the Perciformes (Betancur-R *et al.*, 2017), and several species of the family are an important commercial resource (Hureau and Duhamel, 1980; Duhamel, 1981). Notothenioids are notified to have reduced skeletal ossification (Eastman *et al.*, 2014) but with a normal degree of mineralization for a perciform (Meunier *et al.*, 2018). This light skeleton results of a spongy bone and persisting cartilage in the skull and the appendicular skeleton (Eastman *et al.*, 2014). The bony skeleton of these deep-sea fishes (Meunier *et al.*, 2018) living in cold waters is quite similar to that of other studied Perciformes (Moss, 1965; Meunier and François, 1992; Meunier *et al.*, 2004; Sire and Meunier, 2017; Meunier and Arnulf, 2018; *inter alia*). The skeleton appears normally mineralized and is constituted of tubular acellular bone that shows typical growth marks and, eventually here and there, remodelling of bone (Meunier *et al.*, 2018). Although the scales of the grey notothen have

been used for ageing (Burchett, 1984), to our knowledge their organization and histological structure has never been studied. In the present paper we give the results of a study of the scales of *L. squamifrons*, obtained with histological sections and SEM techniques.

MATERIAL AND METHODS

Material

Lepidonotothen squamifrons: 5 specimens (TL: 273 to 340 mm) from the Kerguelen Islands (Indian sector of the Southern Ocean).

Methods

Histology

The sampled scales were dehydrated in graded series of alcohol till absolute ethanol, transferred to acetone, and embedded in stratyl polymer (chronolite 2195). Transversal and longitudinal sections (150-200 µm thick) were cut

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with an Isomet sawing machine and ground to 50–75 μm in thickness. The sections were microradiographed with a CGR Sigma generator, and then observed under transmitted natural and polarized light with a Zeiss Axiovert 35 microscope.

SEM

A first sample of scales is washed in a diluted hypochlorite solution (5%) for the cleaning of their surface. A more concentrated hypochlorite solution (15%) is used to destroy the unmineralized collagenous areas to visualize the mineralization front of the basal plate on a second sample of scales. Treated scales were then washed in distilled water, and dehydrated in absolute ethanol, before being air dried and gold coated. The upper and deep surfaces of the scales were studied in a JEOL-SEM-35 apparatus.

The spatial organization of the collagenous network of the scale basal plate was processed on scales that were frozen in liquid nitrogen; then they were broken, dried and gold-coated for SEM.

RESULTS – DISCUSSION

Morphology

The scales of *L. squamifrons* are thin imbricated rectangular plates (Fig. 1) that are recognized as elasmoid scales (Bertin, 1958). The scales show four areas: an anterior, two laterals and a posterior bordered by a row of sharp spines the cteni (Fig. 1). The anterior and lateral areas are ornamented by concentric crests the circuli. In the anterior area, circuli show fine denticulations at their tip (Fig. 2A); these denticulations thicken in the vicinity of the focus (Fig. 2B). Such fine denticulated circuli have been described in sev-

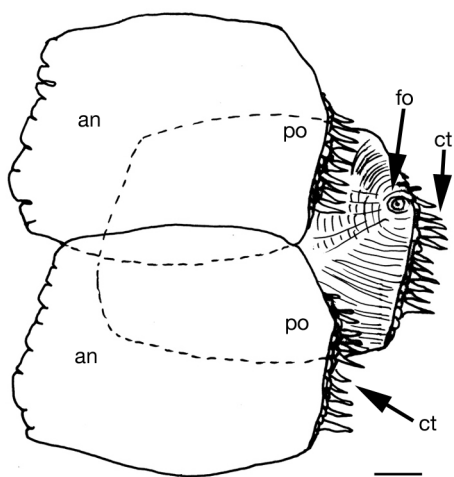


Figure 1. – Drawing of three overlapped scales. The focus (fo) has a posterior location and the surface of the posterior area is reduced. The posterior margin is ornamented by some sharp spines, the cteni (ct). an = anterior side; po = posterior side. Scale bar = 1 mm.

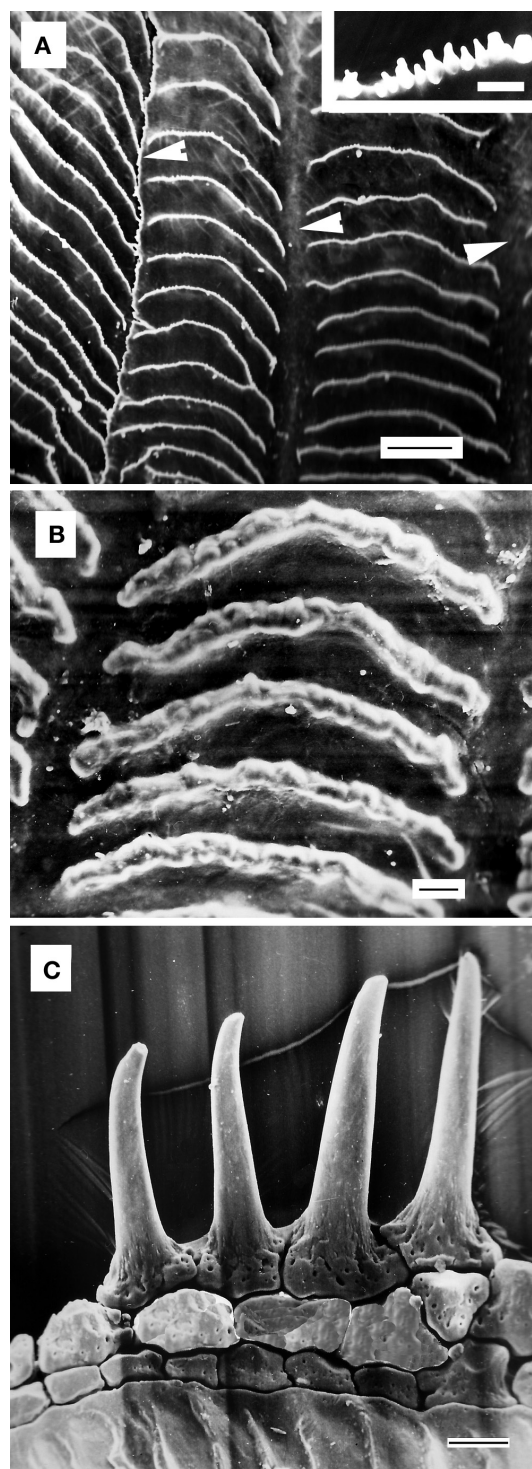


Figure 2. – Superficial view of scales (SEM). A: Detail of the circuli of the anterior area near the anterior margin. We show three radii (arrowheads) and the circuli with some very thin spines at their tip. In the inset, detail of the spines at the tip of the circuli. (scale bar = 7 μm). B: Detail of the circuli of the anterior area near the focus, between two radii. The circuli are thicker than the more anterior ones (compare with Fig. A). C: Detail of the posterior area. Four cteni are seen, the base of which are fit each other and with more ancient ctenial bases. Scale bars: A, C = 100 μm ; B = 25 μm .

eral other teleost fishes (Sire and Meunier, 1981; Zylberberg and Meunier, 1981; Sire and Arnulf, 2000; Zylberberg,

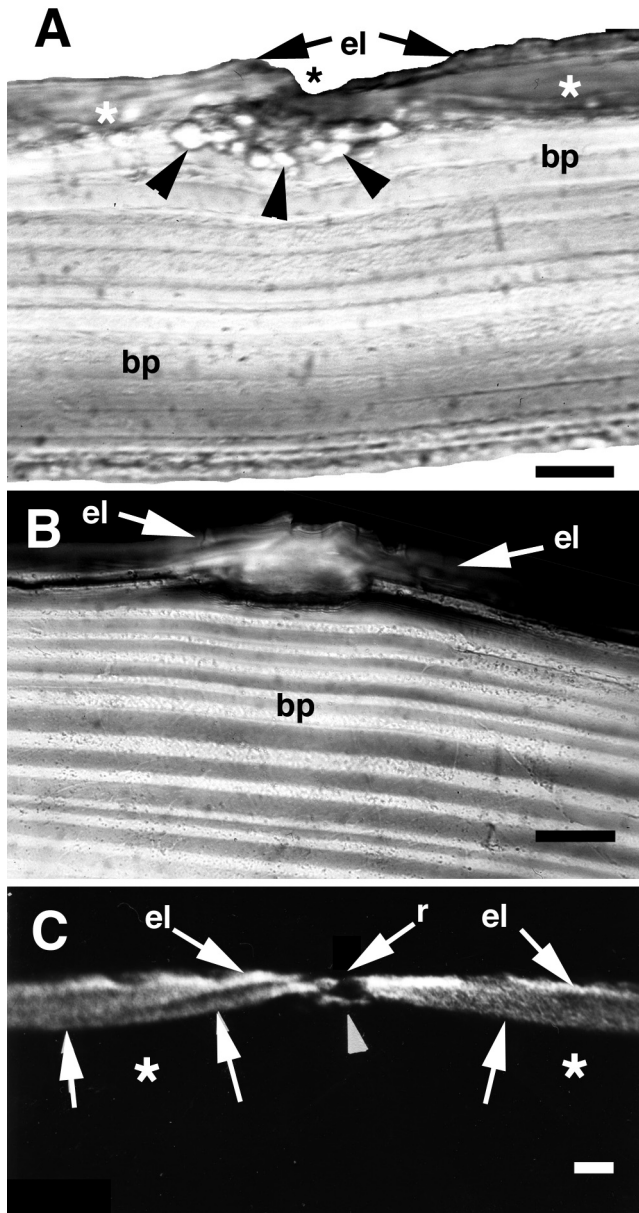


Figure 3. – Transversal ground sections of a scale. **A–B:** Respectively transmitted natural light and polarized light showing the external layer (el) and the successive collagenous layers of the basal plate (bp). **A:** The arrowheads point to mineralized corpuscles of the basal plate under a radius (black asterisk). The white asterisks point to the mineralized part of the basal plate. **B:** In the basal plate (bp) lighted layers alternate with dark layers, indicating an orthogonal plywood-like organization. (el = external layer). **C:** X-ray showing the external layer (el) slightly hypermineralized relatively to the basal plate (white arrows) that is incompletely mineralized (white asterisks). The section crosses a radius (r) that is not mineralized. The arrow-head points to several mineralized Mandl's corpuscles at the bottom of the radius (compare this X-ray preparation to Fig. 3A). Scale bars: A = 10 μ m; B, C = 20 μ m.

2018). The circuli are interrupted by radial grooves, the radii (Fig. 2A, B). The posterior area has a small surface, reduced to ctenial bases of two to three successive ctenial rows; these ctenial bases originating from the distal resorption of cteni fit together and with the new row of cteni (Fig. 2C). So, *L. squamifrons* has ctenoid scales like most notothenioids (Eastman and DeVries, 1982). *Dissostichus* appears to be an exception because their scales have an unmineralized posterior margin that lacks ctenii (Eastman and DeVries, 1981). In some other species that have ctenoid scales (Cockerell, 1913), the number of fit cteni can be significantly higher: in the Cichlidae (Sire and Arnulf, 2000), the Percidae (Zylberberg *et al.*, in preparation), the Soleidae (Roberts, 1993), or the Platycephalidae (Hughes, 1981), for example.

Histology

Each scale is constituted of i) an external ornamented bony layer that is deprived of embedded osteocytes (Fig. 3A), and ii) a pluristratified acellular basal plate (Fig. 3A) incompletely mineralized. The external layer is interrupted at the emplacement of each radii (Fig. 3A). The basal plate is constituted by a number of collagenous layers that are 7–9 μ m thick. The observation of the ground sections with polarized light shows that these layers are alternatively lighted or dark (Fig. 3B). This property indicates that in a given layer the collagenous fibres are parallel to each other and that their directions rotate approximately from an angle of 90° (Meunier and Géraudie, 1980; Meunier and Castanet, 1982; Meunier, 1987–88; Zylberberg *et al.*, 1992). So the collagenous layers form an orthogonal plywood-like organization (see Meunier and Castanet, 1982). In fact, this orthogonal plywood rotates progressively from a small angle (Meunier, 1987–88) and corresponds to the so-called “intermediated” plywood (Meunier and Castanet, 1982).

The scales are incompletely mineralized (Fig. 3C) like in numerous other teleost fishes (Sire and Meunier, 1981; Meunier, 1984, 1987; Meunier and François, 1992; Zylberberg, 2018). The mineralization of the basal plate progresses due to the formation of spherical or ovoid Mandl's corpuscles (Fig. 4A) who progressively fuse to each other (Fig. 4B). Some of them are quadrangular (Fig. 4C) indicating that two ovoid corpuscles localized in two successive collagenous layers are fused. Presence of Mandl's corpuscles in the basal plate of teleost scales is a general characteristic of teleost scales (Mandl, 1839; Baudelot, 1873; Sire and Meunier, 1981; Schönbörner *et al.*, 1981; Meunier, 1984; Zylberberg *et al.*, 1992; Khemiri *et al.*, 2001; and others). The surface of the corpuscles is smooth (Fig. 4). The absence of external ridges on Mandl's corpuscles in scales of the grey notothen indicates that there are no TC fibres crossing the successive collagenous layers of the basal plate (Zylberberg, 2018) contrary to taxa that have TC fibres as the Cyprinidae (Zylberberg *et al.*, 1992) and the Characiformes (Meunier, 1997).

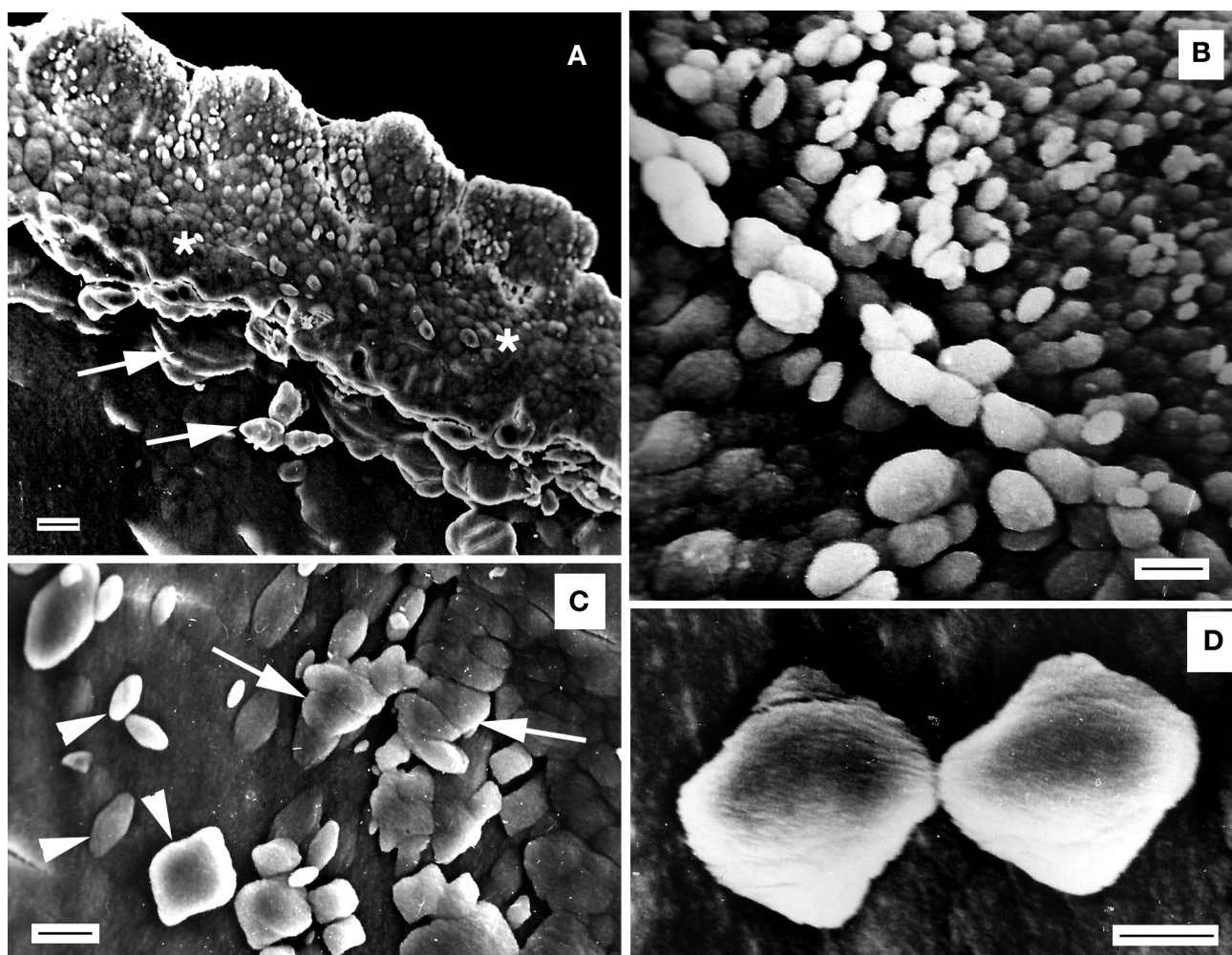


Figure 4. – Mineralizing front of scales (SEM). **A:** Near the margin of the scale the basal plate is mineralized on its whole thickness (white asterisks) and the Mandl's corpuscles are small. At a small distance the basal plate is incompletely mineralized and the Mandl's corpuscles are bigger with an ovoid or a quadrangular shape (arrows). **B:** Detail of spherical and ovoid Mandl's corpuscles. **C:** Detail of the mineralizing front (anterior area) showing on the upper right merging Mandl's corpuscles (white arrows). On the left the mineralizing front is smooth with some Mandl's corpuscles not still merged with the mineralizing front (arrowheads). **D:** Detail of two quadrangular Mandl's corpuscles. Their surface is perfectly smooth. Scale bars: A, B = 20 μ m; C = 10 μ m; D = 5 μ m.

CONCLUSION

The morphological and the histological characteristics of the scales of the grey notothen are similar to those of scales in numerous other Perciformes, such as the Percidae (Zylberberg *et al.*, in preparation), the Cichlidae (Sire and Meunier, 1981; Sire and Arnulf, 2000), the Platycephalidae (Hughes, 1981). Their peculiarities probably play the same functional role especially for hydrodynamism and body protection, as in numerous teleost fishes with ctenoid scales (Burdak, 1976; Vernerey and Barthelat, 2014).

Acknowledgements. – We thanks the “Terres Australes et Antarctiques Françaises” (TAA.) for their help in safely transferring specimens from Réunion Island to the laboratory. We thanks the two

anonymous reviewers for their constructive comments and valuable suggestions.

REFERENCES

- BAUDELLOT M.E., 1873. – Recherches sur la structure et le développement des écailles des poissons osseux. 1^{ère} partie. *Arch. Zool. Exp. Gén.*, 2 : 87-244.
- BERTIN L., 1958. – Écailles et sclérifications dermiques. In: *Traité de Zoologie* (Grassé P.P., éd.), 13: 482-504. Masson, Paris.
- BETANCUR-R., WILEY E.O., ARRATIA G., ACERO A., BAILLY N., MIYA M., LECOINTRE G. & ORTÍ G., 2017. – Phylogenetic classification of bony fishes. *BMC Evol. Biol.*, 17(162): doi: 10.1186/s12862-017-0958-3.
- BURCHETT M.S., 1984. – Age determination of *Notothenia rossii* from South Georgia (Antarctica) using scales and otoliths. *Cybiurn*, 8: 27-31.

- BURDAK V., 1976. – Morphologie fonctionnelle du tégument écaillé des poissons (in Russian, 1979, Kiev). French translation, *Cybium*, 1986, 10 (3, suppl.): 1-147.
- COCKERELL T.D.A., 1913. – Observations on fish scales. *Bull. U.S. Bureau Fish.*, 32: 119-174.
- DUHAMEL G., 1981. – Caractéristiques biologiques des principales espèces de poissons du plateau continental des îles Kerguelen. *Cybium*, 5(1): 19-32.
- EASTMAN J.T. & DEVRIES A.L., 1981. – Buoyancy adaptations in a swim-bladderless Antarctic fish. *J. Morphol.*, 167: 91-102.
- EASTMAN J.T. & DEVRIES A.L., 1982. – Buoyancy studies of notothenioid fishes in McMurdo Sound, Antarctica. *Copeia*, (2): 385-393.
- EASTMAN J.T., WITNER L.M., RIDGELY R.C. & KUHN K.L., 2014. – Divergence in skeletal mass and bone morphology in Antarctic Notothenioid fishes. *J. Morphol.*, 275: 841-861.
- HUGHES D.R., 1981. – Development and organization of the posterior field of ctenoid scales in the Platycephalidae. *Copeia*, (3): 596-606.
- HUREAU J.C. & DUHAMEL G., 1980. – Les poissons et la pêche aux îles Kerguelen. *Cybium*, 4(3): 91-97.
- KHEMIRI S., MEUNIER F.J. & ZYLBERBERG L., 2001. – Morphology and structure of the scales in the gadiformes (Actinopterygii: Teleostei: Paracanthopterygii) and a comparison to the elasmoid scales of other Teleostei. *Cah. Biol. Mar.*, 42: 345-362.
- MANDL L., 1839. – Recherches sur la structure intime des écaïlles des poissons. *Ann. Sci. Nat.*, 2: 337-371.
- MEUNIER F.J., 1984. – Spatial organization and mineralization of the basal plate of elasmoid scales in Osteichthyans. *Am. Zool.*, 24: 953-964.
- MEUNIER F.J., 1987. – Os cellulaire, os acellulaire et tissus dérivés chez les Osteichthyens : les phénomènes de l'acellularisation et de la perte de minéralisation. *Ann. Biol.*, 26: 201-233.
- MEUNIER F.J., 1987-88. – Nouvelles données sur l'organisation spatiale des fibres de collagène de la plaque basale des écaïlles des Téléostéens. *Ann. Sci. Nat., Zool.*, 13^e sér., 9: 113-121.
- MEUNIER F.J., 1997. – Structure et minéralisation des écaïlles de quelques Characiformes de Guyane française. *Rev. Hydrobiol. Trop.*, (1994), 27(4): 407-422.
- MEUNIER F.J. & ARNULF I., 2018. – Some histological data of bone and teeth in the Rift Eelpout, *Thermarces cerberus* (Zoarcidae; Perciform; Teleostei). *Cybium*, 42(1): 83-86.
- MEUNIER F.J. & CASTANET J., 1982. – Organisation spatiale des fibres de collagène de la plaque basale des écaïlles des téléostéens. *Zool. Scripta*, 11: 141-153.
- MEUNIER F.J. & GÉRAUDIE J., 1980. – Les structures en contre-plaqué du derme et des écaïlles des vertébrés inférieurs. *Ann. Biol.*, 19: 1-18.
- MEUNIER F.J. & FRANÇOIS Y., 1992. – Croissance du squelette chez les téléostéens. I. Squelette, os, tissus squelettiques. *Ann. Biol.*, 31: 169-184.
- MEUNIER F.J., LECOMTE F. & DUHAMEL G., 2018. – Some histological data on bone and teeth in the grey notothen (*Lepidonotothen squamifrons*) and in the mackerel icefish (*Champsocephalus gunnari*) (Notothenioidei; Perciformes; Teleostei). *Cybium*, 42(1): 91-97.
- MEUNIER F.J., SORBA L. & BÉAREZ P., 2004. – Présence de tissu osseux acellulaire vascularisé dans les écaïlles élasmoïdes de *Micropogonias altipinnis* (Osteichthyes, Perciformes, Sciaenidae). In 2^e rencontres d'Ichtyologie en France, SFI, *Cybium*, 28 (1 suppl.): 25-31.
- MOSS M.L., 1965. – The biology of acellular teleost bone. *Ann. New York Acad. Sci.*, 109: 337-350.
- ROBERTS C.D., 1993. – Comparative morphology of spined scales and their phylogenetic significance in the teleostei. *Bull. Mar. Sci.*, 52: 60-113.
- SCHÖNBÖRNER A.A., MEUNIER F.J. & CASTANET J., 1981. – The fine structure of calcified Mandl's corpuscles in teleosts fish scales. *Tissue Cell*, 13: 589-597.
- SIRE J.Y. & ARNULF I., 2000. – Structure and development of the ctenial spines on the scales of a teleost fish, the cichlid *Cichlasoma nigrofasciatum*. *Acta Zool.*, 81: 139-158.
- SIRE J.Y. & MEUNIER F.J., 1981. – Structure et minéralisation de l'écaïlle de *Hemichromis bimaculatus* (Téléostéen, Perciforme, Cichlidé). *Arch. Zool. Expér. Gén.*, 122: 133-150.
- SIRE J.Y. & MEUNIER F.J., 2017. – Acellular bone in *Sparus aurata* (Teleostei, Perciformes). A light and TEM study. *Cah. Biol. Mar.*, 58(4): 467-474.
- VERNEREY F.J. & BARTHELAT F., 2014. – Skin and scales of teleost fish: simple structure but high performance and multiple functions. *J. Mech. Phys. Sol.*, 68: 66-76.
- ZYLBERBERG L., 2018. – The elasmoid scales of teleosts: from structure to bioinspired materials. *Cybium*, 42(1): 7-17.
- ZYLBERBERG L. & MEUNIER F.J., 1981. – Evidence of denticles and attachment fibres in the superficial layer of scales in two fishes: *Carassius auratus* L. and *Cyprinus carpio* L. (Cyprinidae, Teleost). *J. Zool. Lond.*, 195: 459-471.
- ZYLBERBERG L., GÉRAUDIE J., MEUNIER F.J. & SIRE J.Y., 1992. – Biomineralization in the integumental skeleton of the living lower Vertebrates. In: Bone (B.K. Hall, ed.), 4: 171-224. CRC Press.

